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(54) Title: METHOD AND APPARATUS FOR ROBOTICALLY PERFORMING SANGER DIDEOXYNUCLEOTIDE DNA SEQUENCING REACTIONS		
(57) Abstract <p>A robotic system for performing nucleic acid sequencing reactions in microtiter plate format. The robot (2) is comprised of a robot arm (5) and its controller (9), robot hands (11, 12, 13) with which it can manipulate microtiter plates with lids and microtiter plates without lids and with which it can perform pipetting in the 5 to 200 microliter range, cooled storage chambers (20) with electronically controllable doors (26) for storage of microtiter plates, cooled storage (23) with an electronically controllable cover for storage of nucleic acid sequencing reagents, heating blocks (14) to incubate the reactions at appropriate temperatures above ambient temperature, storage (15) for boxes of micropipet tips, storage (17) for microtiter plates in which sequence reactions will be performed and software for controlling the operation of the robot during pipetting.</p>		

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**METHOD AND APPARATUS FOR ROBOTICALLY PERFORMING
SANGER DIDEOXYNUCLEOTIDE DNA SEQUENCING REACTIONS**

Reference to Government Grants

Research for this invention was supported in part
5 by National Institutes of Health Grants AR38188 and HL45996.
The United States government has certain rights in the
invention.

Field of the Invention

The present invention relates to the robotic
10 execution of reactions to determine the DNA sequence of
template DNA and more particularly to the robotic execution
of Sanger dideoxynucleotide sequencing reactions.

Background of the Invention

The genetic information for most living organisms
15 is encoded by the molecule deoxyribonucleic acid (DNA). The
genetic code is dependent on the chemical structure of the
DNA. As described below, DNA is composed of four building
blocks that are chemically bonded to one another to form long
polymeric strands. Although DNA is composed of only four
20 building blocks, successive addition of building blocks
rapidly results in a structure with a unique sequence or
combination of building blocks. The possible number of
combinations formed by successive additions of building
blocks rapidly becomes very large as it is the number of
25 blocks to the fourth power.

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Determination of the sequence of an organism's DNA and therefore determination of the sequence of the genes of that organism is essential to the understanding of biology and genetic disease.

5 The DNA of all higher living organisms exists in a double-stranded form that is composed of two complementary, anti-parallel polymeric chains, also called strands (hence double-stranded). Each of the chains is in turn composed of four building blocks called deoxynucleosides. Each
10 deoxynucleoside is composed of a purine or pyrimidine ring structure called a base, a sugar moiety called deoxyribose and a phosphate group. The four deoxynucleosides are adenosine (A), cytosine (C), guanine (G) and thymidine (T). Deoxynucleosides are covalently attached to each other by
15 phosphodiester bonds between the deoxyribose and the phosphate moieties to form the sugar-phosphate backbone of the strand. Because no bonds of the bases are a part of the structure of the backbone of the strand, the bases can be viewed as side-chains of the sugar-phosphate backbone. Each
20 backbone has an orientation because the deoxyribose is an asymmetric molecule and the phosphodiester bonds are formed at hydroxyl groups at the number 3 and number 5 carbons. Conventionally, orientation is designated as 5' to 3' or 3' to 5'. The orientations of the two backbones of the
25 double-stranded DNA molecule are opposite to each other and the strands are said to be anti-parallel.

The genetic information in DNA is encoded by the order or sequence in which the four deoxynucleosides are arranged in the polymeric chains. The order of

30 deoxynucleosides in one chain determines the order of the deoxynucleosides in the opposite chain because the bases specifically interact with bases on the opposite strand to form stable complementary pairs of bases such that A pairs with T and C pairs with G. Because of the base-pairing it is
35 possible to determine the sequence of one strand and infer the sequence of the complementary, anti-parallel strand.

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DNA is synthesized in cells from building blocks that are deoxyribonucleotides. They are similar to deoxyribonucleosides but have a triphosphate moiety instead of the single phosphate found in deoxynucleosides. The two
5 additional phosphates from the triphosphate moiety are released as by-products of the DNA synthesis reaction.

Determination of DNA sequences is of fundamental importance to understand biology and genetic diseases. It is estimated that organisms such as the mouse and man have
10 haploid genomes that contain about 3 billion base pairs.

Advances in the chemistry of the reactions necessary to determine the sequence of DNA have considerably speeded up the process of obtaining DNA sequences as compared to even several years ago. Nonetheless, the reactions are
15 labor-intensive.

Basically, the process of DNA sequencing comprises a series of steps: 1) preparation of the template, 2) performing reactions to generate a series of labeled fragments that begin at a defined point and that are
20 terminated randomly at points where one of the bases occurs in the sequence, 3) separation of the randomly-terminated fragments according to size (length in nucleotides), 4) detection of the separated fragments, and 5) interpretation of the information contained in the pattern of separated
25 fragments. The DNA sequencing reactions that are the subject of this application are those of step 2 above.

Two approaches are currently used to generate the randomly-terminated DNA fragments in step 2 above:

(i) base-specific chemical modification of DNA followed by
30 cleavage of the DNA at the sites of modification (Maxam and Gilbert) and (ii) termination of in vitro synthesized DNA by incorporation of synthetic nucleotide derivatives, called dideoxynucleotides (Sanger). The most widely used of these methods is the Sanger dideoxynucleotide method. It is also
35 more amenable to automation since it involves conditions that are not as extreme, either in terms of physical or chemical

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conditions, as the Maxam and Gilbert chemical cleavage sequencing reactions.

Sanger dideoxynucleotide sequencing consists of annealing a short piece of synthetic DNA (called a primer) to
5 template DNA and synthesis of new DNA by the addition of a DNA polymerase and deoxynucleosides under conditions of salts, pH, buffers and temperature that are appropriate for the DNA polymerase. Addition of dideoxynucleotides at concentrations appropriate for the DNA polymerase causes DNA
10 synthesis to terminate. The termination occurs randomly but is a function of the ratio of the concentrations of the deoxynucleoside and the dideoxynucleotide forms of a particular base. A separate termination reaction is performed for each of the four bases. Each of the four
15 reactions results in a collection of randomly-terminated fragments and together the four collections of fragments represent the sequence information attainable from the template/primer combination. DNA sequencing reactions are critical, time-consuming, labor-intensive and subject to
20 fluctuation in efficiency from person to person.

Accordingly, it would be desirable to provide an apparatus for robotically performing DNA sequencing reactions.

Summary of the Invention

25 It is an object of the current invention to provide an apparatus for robotically performing DNA sequencing reactions in an accurate and reproducible manner. This
~~object, as well as other objects, is accomplished in a~~
robotic system for automatically performing DNA sequencing,
30 comprising (i) a robot controlled by a programmable controller and having an arm mounted for motion in vertical and horizontal planes, (ii) first and second robot hands, said first hand being adapted to carry an microtiter plate, said second hand being adapted for pipetting, a coupling
35 disposed on said arm for attaching each of said robot hands thereto, (iii) heating units adapted to hold a microtiter

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plate, (iv) a plurality of work stations, each adapted to support a microtiter plate, (v) a reagent reservoir holding unit, containing a plurality of reservoirs, and (vi) a controlled temperature storage unit.

5 Brief Description of the Drawings

Figure 1 is a plan view of the overall robotic DNA sequencing apparatus according to the current invention.

Figure 2 is an elevation of the robot shown in Figure 1.

10 Figure 3 is an elevation, partially exploded, of the reagent reservoir shown in Figure 1.

Figure 4 is an elevation of two of the heater blocks shown in Figure 1.

15 Figure 5 is a longitudinal cross-section through one of the heater blocks shown in Figure 4.

Figure 6 is an elevation of a microtiter plate storage unit and work station.

Figure 7 is a plan view of a microtiter plate.

20 Figure 8 is an elevation showing the multichannel pipet suspended above a microtiter plate.

Figure 9 is a schematic diagram of a peristaltic pump for one of the tip washing stations shown in Figure 1.

Figure 10 is a flow chart of the pipet tip control method according to the current invention.

25 Description of the Preferred Embodiment

Figure 1 shows an overall view of the robotic DNA sequencing system 1 of the current invention. The system includes a robot 2 controlled by a controller 9. The controller 9, which contains a programmable micro-processor, 30 interfaces with a personal computer 10 that allows the operator to initiate and otherwise supervise the operation of the system. However, once initiated, the system is designed to operate automatically until all of the DNA samples have been processed. Such robots are commercially available and 35 may be purchased from the Zymark Corporation. As shown in

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Figure 2, the robot 2 is comprised of an arm 5 slidably mounted for horizontal motion in a housing 29. The housing 29 is slidably mounted for vertical motion on a support shaft 6 that is attached to a rotatable swivel plate 4 supported on a base 3. This arrangement provides the robot 2 with a cylindrical work space defined by rotation through approximately 376°, a 35 cm range of movement in the vertical direction, and a radial extension of about 65 cm in the horizontal direction.

10 A coupling 7 is disposed at the end of the arm 7 and allows a variety of general and special purpose "hands" to be installed onto the arm 5. In the current invention, these hands include a light duty hand 11, a heavy duty hand 12, and a specialized multichannel pipet hand 13. This last
15 hand is shown in detail in Figure 8.

Referring again to Figure 1, the system also includes six heater blocks 14, various pipet wash stations 18, a pipet tip station 15, microtiter plate work stations 16, microtiter plate storage units 17, a reagent reservoir
20 holding unit 21, pipet storage units 19, microtiter plate controlled temperature storage units 20, and a pipet disposal station 30.

As shown in Figure 3, the reagent reservoir holding unit 21 is comprised of a cooling tank 23 to which cooled
25 water is circulated from a cooling and pumping unit 42 via hoses 41. In the preferred embodiment, the cooling water is maintained at approximately 4°C. The tank is capable of holding and cooling eight reagent reservoirs 22. In addition, a lid 24 is provided that is operated by a
30 pneumatic operator 25 electrically controlled by the robot controller 9.

The heating blocks 14, two of which are shown in Figure 4, employ heating elements (not shown), which may be resistance heaters. As shown in Figure 5, each heating block
35 14 has a sculptured upper surface in which ninety six troughs 31 are formed. The troughs have approximately the same size, shape and layout as the outside surfaces of the wells 40 of a

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plastic standard microtiter plate 34, shown in Figures 7 and 8. In addition, the heating blocks 14 have a flat support surface 32 adjacent the sculptured upper surface that is adapted to stably support the edges of a microtiter plate 34.

5 The heating blocks 14 also feature beveled edges 33 that are adapted to guide the microtiter plate onto the sculptured surface. In the preferred embodiment, the heating blocks 14 maintain the contents of a microtiter plate 34 at approximately 65°C.

10 As shown in Figures 7 and 8, standard ninety six well microtiter plates 34 are utilized, with each well having a capacity of about 300 μ l. The wells 40 are arranged in twelve columns, with eight wells in each column. In the preferred embodiment, only the first seven columns C1 to C7

15 are utilized.

The arrangement of the microtiter plate storage unit 17 and work station 16 are shown in Figure 6. As can be seen, each storage unit 17 is comprised of a number of compartments 36, each of which is adapted to hold a single

20 microtiter plate 34 with its lid 35. The microtiter plate work stations 16 comprise a flat work surface 35 adapted to support an microtiter plate 34.

As shown in Figures 1, 4, and 6, pipet wash stations 18 are disposed adjacent the heating blocks 14 and

25 microtiter plate work stations 16. The pipet storage units 19 are each comprised of a vertical array of storage compartments, similar to the microtiter plate storage unit 17, that are adapted to hold boxes of plastic disposable

~~pipet tips 50 prior to their insertion onto the multichannel~~

30 pipet hand 13, as shown in Figure 8. The temperature controlled storage units 20 have doors 26 that are electrically operated by the robot controller 9. The temperature controlled storage units 20 have a vertical array of storage compartments each of which is adapted to hold two

35 pairs of microtiter plates, each pair consisting of one microtiter plates stacked on another. The two pairs are stored next to one another. In the preferred embodiment, the

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storage units 20 are maintained at a temperature of 6°C by the use of circulating cooled water. In addition, the storage units 20 may be equipped with a vacuum forming unit if it is desired to dry the reagents.

5 In the DNA sequencing method according to the current invention, Sequenase (available from US Biochemicals) Taq DNA polymerase (available from Perkins-Elmer Cetus) and Bst DNA polymerase (available from Bio-Rad) may be used for dideoxynucleotide DNA sequencing, although in principle
10 almost any DNA polymerase may be used. Reaction conditions are based on the two-step reaction in which the initial extension of the primer and incorporation of radiolabeled nucleotide is performed as a separate step from the termination step. Radiolabeled dATP (³⁵S-dATP; ³³P-dATP)
15 (available from New England Nuclear) and solutions of deoxynucleosides and dideoxynucleotides (available from Boehringer Mannheim Biochemicals) may be used for this purpose. Optimal ratios of dideoxynucleotide to deoxynucleoside (Boehringer Mannheim) may be determined
20 empirically. Products of sequencing reactions are separated on 6% DNA sequencing gels (Sequagel 6%; National Diagnostics) that are fixed in 10% methanol-10% acetic acid and dried under vacuum, and exposed to X-ray film to produce autoradiographs.

25 In the preferred embodiment, the system allows thirty micro-titer plates 34 and lids 35 to be stored at room temperature prior to sequencing and a maximum of forty plates with completed sequencing reactions to be stored in the cooled storage units 20. Therefore, the robot 2 can perform
30 a maximum of 240 sequencing reactions (30 plates, 8 reactions per plate) in a single run. The system can, however, be configured to allow ambient, as well as cooled storage, of a maximum of sixty microtiter plates and, therefore, will allow a maximum of 480 reactions to be performed in a single
35 unattended run.

Sequencing reactions, as performed manually, impose significant constraints on automation. The chief constraint

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is to pipet, reproducibly and precisely, volumes as small as 0.5 μ l. However, pipetting, reproducibly and precisely, a volume of 0.5 μ l is beyond the capacity of most automated pipetting systems.

- 5 One solution to this problem involves the use of dried reagents since they allow the use of larger volumes. The dried reagents in the target wells occupy no volume, therefore, the volume that is transferred can be larger by the volume that is normally occupied by the dried reagents.
- 10 Use of dried reagents allow the use of volumes that for the smallest volumes are in the range of seven microliters, volumes that can be pipetted reproducibly and precisely by many automated systems. Consequently, the invention may be practiced by performing sequencing reactions using reagents
- 15 dried under vacuum onto microtiter plates. In this case, the system would include cooled storage units 20 that could be placed under vacuum to dry down the reagents on microtiter plates. Such a system must: operate without intervention after the initial setup, have cooled storage that
- 20 incorporated a robotically controlled lid for reagents, have cooled storage that could be placed under vacuum for microtiter plates, have several (preferably six or more) heat blocks sculpted to fit microtiter plates, be capable of manipulating microtiter plates and be capable of reproducibly
- 25 pipetting volumes as small as seven microliters from microtiter plate wells containing as little as twelve microliters.

However, according to the current invention, the problems of pipetting small volumes may also be overcome by

30 the use of surfactants along with a novel method of controlling the movement of the multichannel pipet hand 13 into the microtiter plate wells 40, thereby allowing the use of reagent solutions.

One of problems associated with pipetting small

35 volumes of solutions is the tendency for droplets to bead up on the microtiter plates. The beading frequently results in failure to recover the desired volume from a well 40.

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Experiments to decrease the surface tension with non-ionic detergents indicate that a final concentration of 0.02% was adequate to prevent beading on polystyrene microtiter plates 34. However, the inventors have found that a concentration of 0.025% non-ionic detergents is not always adequate to prevent beading on the surface of the polypropylene inserts used in the reagent reservoirs 22. To consistently prevent beading of the solutions on the polypropylene surface it is necessary to increase the concentration of non-ionic detergents to at least 0.05%. Inclusion of the non-ionic detergents in all the reagents makes it possible for the robot to recover, reproducibly and precisely, seven microliters out of a total of 12 μ l in each well 40 of a column of eight microtiter plate wells. In addition, it allows the robot to recover almost all of the reagents from the reservoirs 22, leaving as little as 20 μ l.

According to the method on the current invention, the robot controls the movement of the multichannel pipet hand 13 so that the pipet tips 50 touch the sides of the wells 40 while dispensing, so as to wipe the tips. Touching the sides provided a surface for the drops to run down, thereby preventing the solution from beading back onto the tip surface, since such beading would prevent all of the volume aspirated from being dispensed. Wiping of the tips resulted in increased reproducibility of the dispensed volume.

In the preferred embodiment, the operating system for the robot controller 9 utilizes an interpreted language with ~~macro-like modules that can be nested to seven layers.~~

Standard programming provided by the robot supplier may be used to control some basic functions that involve the coordination of power-and-event controller switches, such as the opening and closing of doors 26 of the temperature controlled storage units 20. However, software necessary to adequately control the robot's ability to pipet small volumes of about 7 μ l volumes or less from source volumes of about 12 μ l has not heretofore been available.

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Although the multichannel micropipet hand 13 itself is capable of pipetting small volumes reproducibly and precisely if the pipet tips 50 are properly located within the well, the dexterity of the robot 2 in moving the multichannel micropipet hand 13 into the well 40 is crucial to accuracy. Figure 8 shows the multichannel micropipet hand 13 suspended above a microtiter plate 34 just prior to insertion of the tips 50 into the wells 40 for aspiration. The inventors have found that accurate pipetting of small volumes from small source volumes requires that the pipet tips 50 reach to just above the bottoms of the microtiter plate wells 40. If the tips 50 are too high, they will not be immersed in the liquid for the last few microliters. If the tips 50 touched the bottoms of the wells with excessive force, however, the holes of the tips will be blocked and the recovery variable.

Unfortunately, because the robot's reach (i.e., from about 34 cm to about 70 cm depending on the hand) there is some sway or movement at the extremity of the arm 5. As a result, it is not possible to program the robot's movement to within the required sub-millimeter precision using absolute coordinates. This problem is solved in the current invention by programming the robot 2 so that it "senses" contact between the pipet tips 50 and the bottoms of the wells 40. Accordingly, the robot is equipped with force sensors 60, which may be of the strain gage type, that are coupled to the arm 5, as shown in Figure 2. The sensors 60 provide a measure of the force exerted on the arm in arbitrary units. ~~The feedback from the sensors 60 enables the arm 5 with the~~ micropipet hand 13 to sense when the tips 50 make contact with the bottoms of the wells 40. After the tips 50 make contact, the height of the arm 5 is increased slightly to prevent the blockage of the tips.

Unfortunately, the force sensors 60 lack precision and report variable readings depending on the recent movements of the arm 5. Specifically, the inventors have found that the value obtained from the force sensor 60 with

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the hand 13 is hanging freely is higher when the hand had recently been moved upward than when it had recently been moved downward. Also the sensitivity of the sensor 60 is insufficient to detect contact by an absolute measure.

- 5 However, these limitations are overcome in the current invention by a novel pipet tip movement control method, a flow chart for which is shown in Figure 10, for sensing when the tips 50 are at the appropriate location in the bottom of the wells 40 of a microtiter plate 34 for proper aspiration.
- 10 This location, defined as the "contact point," is that location in which the pipet tips 50 almost touch the microtiter plate 34 so that the tips are not pressed against the bottoms of the wells, which would impeded flow into them, nor are they so far above the bottoms of the wells as to
- 15 prevent complete aspiration. The inventors have found that when the contact point is achieved, the microtiter plate can not be easily lifted from the sculptured surface of a heat block 14 but that the tips, which are somewhat flexible, do not show any signs of being subjected to force, such as
- 20 bowing out. In addition, it has been found that when the contact point is achieved, it is possible to fractionally lift the microtiter plate off the heating block 14 if the height of the hand 13 were increased by 0.2 mm.

- According to the pipet tip movement control method,
- 25 the sensing of bottoms of the wells is refined by moving the pipet tips 50 toward the bottoms of the wells in a series of increasing smaller decrements in the height of the pipet tips above the wells 40, accomplished by a logic module referred to as "bottom sensing" in Figure 10. Since the force sensed
- 30 by the sensor 60 includes a force component attributable to the weight of the freely hanging multichannel pipet hand 13, the measured force will decrease when the pipet tips 50 contact the bottoms of the well because the wells will then be supporting at least a portion of the weight of the hand.
- 35 Therefore, each incremental decrease in height is alternated with a decision step that tests whether the force on the pipet tips, as measured by the sensor 60, is sufficiently

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small to indicate that the tips have contacted the bottoms of the wells.

Because of the insensitivity of the force sensors 60, described above, it is necessary that the force on the pipet tips 50 be sufficiently great to bow the tips outward in order to be assured that contact with the bottoms of the wells has occurred. Moreover, the point at which the tips are bowed is achieved incrementally to provide reproducibility. Thus, the value that the measured force must be below, to satisfy the criteria for "bottom sensing" according to the method, is set so that at such a value of measured force, the pipet tips 50 can be expected to be bowed outward. However, reliable aspiration can not be achieved with the pipet tips pressing against the well bottoms with such force since their inlets are apt to be blocked by the contact. Consequently, after "bottom sensing," as determined above, is established, the height of the hand 13 is increased slightly until the measured force is sufficiently great to indicate that the pipet tips are almost contacting the bottoms of the well but without being subjected to appreciable force so that the hand 13 is essentially again hanging freely -- this condition being the "contact point."

Of course this increase in height following bottom sensing must be sufficiently small or complete aspiration will not be achieved. Thus, according to the current invention, the raising of the hand 13 following bottom sensing is also done incrementally, in a logic module referred to as "contact point sensing" in Figure 10. The contact point sensing module lifts the arm in very small increments, the first of which is equal to the product of a small constant k times the number of loops, $TRYA$, through the bottom sensing module that were necessary to satisfy the "bottom sensing" condition. This incrementing is continued until the detected force is equal to the force sensed when the hand 13 was hanging freely, thereby indicating that the pipet tips are not pressing into the wells such that flow into them may be impeded.

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Although the force with a freely hanging hand is measure just after the hand was suspended over the wells -- that is, following a downward movement of the hand -- due to the "history" phenomenon previously discussed, that measured
5 force, defined as F_{ref} in Figure 10, will be greater than the force measured with a freely hanging hand that has been incremented upward during execution of the contact point sensing module. Consequently, a constant, Δf_2 , is subtracted from F_{ref} to correct for the history effect in determining
10 when the hand is hanging freely so that the contact point can be considered to have been reached in the contact point sensing module. In the preferred embodiment, the constant Δf_2 is equal to the difference in the force measured with a freely hanging hand between a measurement taken after the
15 hand was moved up and a measured taken after the hand was moved down and need be established only once to account for the peculiarities of the particular robot system.

If the pipet tips fail to touch the bottoms of the wells after executing the bottom sensing module four times
20 (three loops through route A in Figure 10), the bottom sensing module is attempted from the beginning one more time (route B in Figure 10). If they fail to touch the bottoms of the wells after an additional four passes through the bottom sensing module, the robot is halted.

25 Once the height necessary to achieve the "contact point" is determined, its value is stored and reused each time the hand 13 returns to that particular microtiter plate to aspirate another column of wells. Thus, the point of
~~contact is determined only once for each set of eight~~
30 sequencing reactions performed on a given microtiter plate. In the preferred embodiment, contact point sensing is determined for each microtiter plate when the column of wells containing the extended primer and template DNA together with the other reagents including the DNA polymerase are aspirated
35 just prior to dispensing the reaction to the four termination mixes.

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With specific reference to Figure 10, the multichannel pipet control method of the current invention may be explained as follows. In step 100, the values of the counters TRYA and TRYB are zeroed after the hand 13 has moved
5 into position above the microtiter plate as shown in Figure 8. Note that to overcome the variability due to the effect of the recent history of the arm's movement on the force sensor 60 output, the force sensor is standardized by always moving toward and traversing the microtiter plate 34 in a
10 similar manner -- e.g., moving radially toward and across the plate -- prior to beginning the method.

With the hand 13 suspended above the microtiter plate as shown in Figure 8, the force F as sensed by the force sensor 60, which is equal to the force from a freely
15 hanging hand after the hand has been moved downward, is measured and stored as F_{ref} in step 101. In step 102, the height H of the multichannel pipet hand 13, which, as shown in Figure 8, is initially poised just above the wells 40 of the microtiter plate 34, is incremented downward by a
20 relatively large increment Δh_0 . In step 103, the force F from the sensor 60 is measured again and it is determined whether or not this force is less than $F_{ref} - \Delta f_1$, Δf_1 being an empirically determined constant based on the particular robot, thereby indicating that bottom sensing has occurred.
25 If it is less, the contact point sensing module is executed, as explained below. If it is not, then in step 104 the height H is incremented downward by a second increment Δh_1 , Δh_1 being less than Δh_0 , so that a smaller, finer increment
is used in the second step down.

30 In step 105, the force F from the sensor 60 is measured again and it is again determined whether or not this force is less than $F_{ref} - \Delta f_1$. If it is less, the contact point sensing module is executed. If it is not, then in step 106 the height H is incremented down by a increment Δh_2 , Δh_2
35 being less than Δh_1 , so that a still smaller, finer increment is used in the third step down.

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In step 107, the force F from the sensor 60 is measured again and it is determined whether or not this force is less than $F_{ref} - \Delta f_1$. If it is less, the contact point sensing module is executed. If it is not, then in step 108
5 the height H is incremented down by a increment Δh_3 , Δh_3 being less than Δh_2 so that the bottom of the well 40 is approached with increasingly finer increments, and in step 109, $TRYA$ is increased by one. In step 110, it is determined whether or not $TRYA$ is less than or equal to three. If it
10 is, then steps 103 to 110 are repeated, unless bottom sensing is achieved at some point through the module.

If $TRYA$ is not less than or equal to three, then in step 111, $TRYB$ is increased by one and $TRYA$ set to zero. In step 112, the height is increased by the amount of the
15 original decrement Δh_0 so that further execution of the bottom sensing module is begun with a height that is less than the original height by $4 \times (\Delta h_1 + \Delta h_2 + \Delta h_3)$. In step 113, it is determined whether or not $TRYB$ is less than two. If it is, then steps 101 to 113 are repeated. If it is not, then
20 the operation is halted and the problem investigated.

The contact point sensing module is initiated in step 115 by raising the height H of the arm by $H + (TRYA \times k)$, k being an empirically determined constant based on the particular robot. Thus, the amount by which the height of
25 the hand 13 is raised -- that is, the increase in height necessary to ensure that the tips 50 are not pressing against the wells with sufficient force to impede flow yet to also ensure that the tips are not moved so far from the bottom of the well that aspiration will be incomplete -- is a function
30 of the number of times the group of incrementing steps 104 to 108 were performed during the execution of the bottom sensing module that attained the contact point.

In step 116, the force F is measured again and it is determined if it is greater than $F_{ref} - \Delta f_2$, Δf_2 being an
35 empirically determined constant to correct for the history effect, as previously discussed. If it not, then the height H is raised again by the increment Δh_3 and steps 11 to 119

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are repeated. If it is greater, then the value of H_{ref} is set to H so that repetitions of the aspiration on the same microtiter plate can be repeated using the same height value determined above during the first aspiration on a particular
5 microtiter plate. Lastly, in step 119 the aspiration of the contents of the wells 40 in the particular microtiter plate column are aspirated.

Empirically determined constants for height differences are about as follows: Δh_0 is slightly less than
10 the height difference measured between the bottoms of the micropipet tips and the bottoms of the microtiter plate wells when the micropipet tip hand is in the position suspended over the wells. It is dependent on the definitions for the positions at and above the heating blocks 14, taught to the
15 robot. The other empirical constants, Δh_1 to Δh_3 , are in the sub-millimeter range, usually less than 0.1 mm, and initial values are used such that $\Delta h_2 = (1.7 \times \Delta h_3)$, and $\Delta h_1 = (2.5 \times \Delta h_3)$. For the empirically defined force constant Δf_1 , the starting value is about equal to Δf_2 as defined above. All
20 empirical values are refined by trials.

As can be readily appreciated, those skilled in the programming arts can readily program the logic discussed above and shown in Figure 10 into a microprocessor of a robot controller, such as controller 9 shown in Figure 1, so that
25 the microprocessor provides the capability for performing the logic steps shown in Figure 10.

One consequence of performing parallel reactions staggered by five minutes is that the time required to change micropipet tips 50 becomes rate limiting. To overcome the
30 limitation, tip washing stations 18 are disposed adjacent those positions where the robot performs pipetting operations. For this purpose, plastic inserts are used similar to those used for the cooled reagent reservoir 22, except that the tip washing plastic inserts have a nominal
35 volume of 38 ml, whereas the cooled reagent reservoir plastic insert have a nominal volume of 18 ml. The tip washing stations 18 are mounted such that they are radially in-line

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with the microtiter plate and their upper edge is at the same height as the upper edge of a microtiter plate at the particular pipetting station, as shown in Figures 4 and 6.

When the tip washing station 18 is completely
5 filled, it is possible to wash the tips without any additional adjustments with regard to rotational or vertical movement. A system driven by a peristaltic pumps 61 and 62, shown in Figure 9, may be used to replenish the water in the tip washing stations 18 so as to maintain the volume at a
10 constant level and prevent the accumulation of reagents during long runs may also be incorporated into the system. As shown in Figure 9, in addition to the pumps 61 and 62, the system includes a source reservoir 63 and a waste reservoir 64, each in flow communication with a tip washing station 18
15 by means of hoses 65 and 66.

Tips are washed by aspirating and expelling 50 μ l several times. To ensure that little or no water is transferred to the next reaction, with a resulting dilution of the reagents, the last expulsion of the washing solution
20 is performed slowly while the tips are touching the side of and upper edge of the tip washing station to prevent beading of the water back onto the tips.

Another problem encountered when the microtiter plate format is used to facilitate automation of DNA
25 sequencing according to the current invention is that evaporation of reagents occurs during the various heating steps in the reactions. This problem is solved in the current invention by the addition of non-ionic detergents containing water to compensate for volume loss due to
30 evaporation, as explained further below.

The system can perform 240 reactions in a single unattended run of seven hours. However, the robot configuration can be altered to allow 480 reactions to be performed in an unattended run of fourteen hours. Therefore,
35 if a daytime run of 240 reactions in seven hours is combined with a nighttime run of 480 reactions in fourteen hours, a total of 720 reactions could be performed per day.

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The process of DNA sequencing consists of a series of steps: 1) preparation of the template, 2) performing reactions to generate a series of labeled, base-specific, randomly terminated fragments, 3) separation of the randomly-terminated fragments according to length, 4) detection of the separated fragments, and 5) interpretation of the information contained in the pattern of separated fragments. Automation of the steps results in greater throughput of sequences. Automation of all of the steps is essential for improved throughput in a single laboratory. Automation of the reactions necessary to generate the series of fragments is discussed in detail below.

In addition, preparation of DNA template can be performed with instruments such as the Biomek 1000 work station (available from Beckman) or Autogen 540 (available from Autogen). Detection of the separated fragments and interpretation of the information can be performed with fluorescence-based sequence detection systems coupled to computers such as the ABI-370 (available for Applied Biosystems), the A.L.F. (available from Pharmacia), or autoradiograph-based computer graphics systems such as that supplied by Bio-Rad or the Bio Image (available from Millipore) scanners and analysis systems. For further analysis of the information, many computer software packages are available.

The DNA sequencing method according to the current invention is begun by manually loading the reservoirs 22 with reagents. ~~Enzyme plus label is placed in reservoir no. 1,~~ terminations G, A, T and C, respectively, in reservoirs nos. 2-5, the STOP buffer in reservoir no. 6 and water in reservoir no. 7. Boxes of pipet tips 50 are manually loaded into the pipet storage units 19 and the DNA sample is manually deposited into the wells 40 of up to three microtiter plates 34. Because the samples are used eight at a time, one column at a time, and there are twelve columns on a microtiter plate 34, and because the maximum number of

- 20 -

samples that can be processed in the preferred embodiment is 240 samples, up to three microtiter plates 34 containing samples may be placed on work surfaces of the robot. Two of the three microtiter plates may have all ninety six wells
5 forty completely filled with samples and the third may have up to forty eight wells 40, wells in column 1 through column 6, filled.

In the preferred embodiment, more than one microtiter plate is used to increase the number of reactions
10 performed in a set time. This is accomplished by programming the robot to perform two sets of reactions that are out of phase by five minutes, therefore, samples are deposited into the sample microtiter plates so that there are always even numbers of columns of samples. The reaction conditions for
15 Bst DNA polymerase (see below) are modified such that each of the basic steps (i.e., heating or denaturation, annealing, labeling/extension and termination) are performed for about equal lengths of time -- i.e., in blocks of about five minutes. Since all the sub-parts of the reactions are
20 performed in blocks of the same length of time, two sets of reactions can be sequenced so that they are out of phase by one block (five minutes). That is, when the first sample is undergoing annealing, the second sample is undergoing denaturation, etc. In this manner, two sets of reactions can
25 then be performed in twenty seven minutes. To accommodate the maximum number of plates in the cooled storage units 20, the robot 2 is programmed to stack one microtiter plate 34 on top of another prior to moving them to one of the temperature controlled storage units 20.

30 Once manual locating has been completed, automatic operation is then initiated. Interchanging the light weight and heavy weight hands 11 and 12 and the multichannel pipet hand 13, as required, the robot 2 steps through the sequence of operations programmed into the controller 9, performing
35 the reactions on a pair of samples for each cycle, as explained above, and performing additional cycles until all of the samples have been sequenced. The light weight hand 11

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is used to move boxes of micropipet tips 50 from the micropipet tip storage units 19 and to move microtiter plates 34 from microtiter plate storage units 17. The heavy duty hand is used to manipulate microtiter plates 34 and lids 35, 5 when they are on the heating blocks 14 and especially when the microtiter plates 34 with lids 35 are stacked on one another and moved to the temperature controlled storage units 20.

In the first step, the robot 2 using the light weight hand 11 removes a box of tips 50 from the tip storage 10 19 and places it on the tip work station 15. All of the sample plates are first placed in the temperature controlled storage unit 20 using the heavy duty hand 12. The first microtiter plate 34 containing samples is then placed on a 15 work station 16. Next, using the light duty hand 11, two empty microtiter plates 34 are removed from a microtiter plate storage unit 17 and placed on each of two of the heater blocks 14 in the outer row of heating blocks and the lids 35 of the microtiter plates are removed and placed on an 20 adjacent work station 16 or a heating block 14. Note that the system 1 incorporates an outer row of heating blocks 14, in addition to an inner row, so that heating can be carried out at two temperatures. However, in the preferred embodiment of the method, heating is only necessary at one 25 temperature so that the outer row of heating blocks 14 function merely as work stations and do not produce heat. The lid of the microtiter plate containing samples on work station 16 is removed and placed on an adjacent work station 16.

30 Using the multichannel pipet 13, the robot aspirates one column of wells 40 from the sample containing microtiter plate on the work station 16 and dispenses them into the first column of one of the empty microtiter plates on the outer row of heating blocks. The tips 50 are then 35 washed in the tip washing station 18. These steps are repeated so that the first column of the second, previously

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empty, microtiter plate on the outer row of heating blocks is also filled with sample.

Next, the lids of the microtiter plates on the heating blocks are replaced and one of the microtiter plates from the outer row of heating blocks is placed on an active heating block 14 in the first row, whereupon it is heated for five minutes at 65°C to complete the denaturation step. The lid of the microtiter plate that contained samples is replaced and the microtiter plate is then returned from work station 16 to the storage unit 20 if there are still samples remaining on the microtiter plate. If no samples are remaining, the microtiter plate is disposed of to free up the work station 16. The other microtiter plate from the outer row of heating blocks is placed in the storage unit 20. As previously discussed, these two microtiter plates are processed so that one of the basic steps on each is followed by one of the basic steps on the other. However, for simplicity, the method will be described from beginning to end with respect to only one microtiter plate without interrupting the explanation to discuss the out of phase reaction being performed on the second microtiter plate.

The microtiter plate containing the now denatured sample is then placed on a work station 16 where it is held at room temperature for five minutes to complete the annealing step. Its lid is removed and placed on an adjacent work station 16.

During the time that the annealing is taking place while the plate the microtiter plate is at room temperature, the reagents are aspirated from the reservoirs 22 and dispensed into the microtiter plate containing the denatured sample. First, the STOP buffer is dispensed into column no. 6, followed by tip washing in the tip washing station 18. The water is then aspirated and dispensed from its reservoir 22 into column no. 7 of the microtiter plate and the tips are again washed. In sequence, the terminations G, A, T, and C are then aspirated from their reservoirs 22 and dispensed into column nos. 2-5 of the microtiter plate, with the tips

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being washed between each operation. Following these steps, the tips 50 are disposed of in the tip disposal unit 30 and a new set of tips from the tip work station 15 are placed on the multichannel pipet 13.

- 5 Next, the enzyme/label is aspirated from its reservoir 22 directly into the DNA sample in column no. 1 of the microtiter plate. The lid of the microtiter plate is replaced and the microtiter plate is then placed on one of the active heating blocks 14 to begin the five minute label
- 10 and extension step while being heated to 65°C. After five minutes the lid of the microtiter plate is removed to an adjacent heating block 14 and the reaction volume in column 1 is supplemented with the water from column 7 by aspirating water from column 7 and dispensing into column 1. The five
- 15 minute termination step is then begun by sequentially pipetting portions of sample from column no. 1 of the microtiter plate into columns nos. 2-5 containing the G, A, T and C terminations. The lid of the microtiter plate is replaced for the five minute incubation. After five minutes,
- 20 the lid is removed to an adjacent heating block and portions of the STOP buffer are then aspirated from column no. 6 into each of columns nos. 2-5 to stop the reactions. After the addition of the STOP buffer to the termination reactions, the lid is replaced and the microtiter plate is moved to an
- 25 adjacent heating block that is at room temperature while the reactions in the other microtiter plate in the pair is completed. This microtiter plate is then stacked on top of the other microtiter plate in a pair, which by now has also
-
- 30 ~~been processed in the sequential, out of phase method~~ previously discussed, and both are stored in the storage unit 20 while the second pair of samples are processed. Processing and storing of successive pairs of samples is continued until all of the samples are sequenced. When the micropipet tips 50 in a box in the tip station 15 are
- 35 depleted the empty box is disposed of by returning it to a rack in a tip storage 19. A new box of micropipet tips 50 is then moved from the tip storage 19 to the tip station 19.

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Following sequencing, the samples are placed on a sequencing gel the gels are electrophoresed, fixed, dried and X-rayed film is exposed to it to generate an autoradiogram to identify the DNA sequence using techniques well known in the art.

Sequenase is an enzyme that has become a standard for sequencing in many laboratories. To compare the sequencing reactions performed by the robot with those performed routinely in other laboratories, the reaction conditions for Sequenase were modified for use on the robot. DNA and primer were heated to 65 °C for five min., the primer was annealed to the DNA at room temperature for four min., the enzyme-labeling mix was added and the reaction incubated for four minutes at room temperature, the extension-reaction products were dispensed into the termination mixes and the reactions were incubated at 37 °C for five min. Sequencing reactions were stopped by the addition of STOP buffer. Because of the constraints on pipetting, the volumes of the reactions had to be increased. When microtiter plates were incubated at 65 °C, there was loss of volume due to evaporation even though the plates were covered with lids at all times except during pipetting. Therefore the annealing volume was increased to 16 μ l as compared with 10 μ l in the standard protocol. After annealing the effective volume was about 10 μ l. The buffer in the annealing mix was adjusted to be correct for the effective volume. The enzyme-labeling mix was essentially identical to the standard protocol except that non-ionic detergents were added to a final concentration of 0.02% and the volume of enzyme dilution buffer was about one-third of the standard protocol. After the labeling-extension reaction was complete, the volume was increased by the addition of 9 μ l of water to about 25 μ l. Five microliters of the labeling-extension reaction was dispensed to each of the 5 μ l termination mixes. The reaction was terminated by the addition of four μ l STOP buffer.

The sequences generated were comparable to those generated by an individual experienced at sequencing.

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Furthermore, there was little if any variation from well to well, and from plate to plate for a single template.

Sequenase is, however, labile. The manufacturer recommends not storing the diluted enzyme for more than 60 min. at 4 °C. To achieve the goal of long periods of unattended operation, it was essential to use an enzyme that was stable in solution at 4 °C at the working dilution. The DNA polymerase from *Bacillus stearothermophilus* (Bst DNA polymerase) is reported to be extremely stable and is functional for sequencing even after fourteen days of storage at room temperature. Therefore, we investigated its use for the robot. The sequencing conditions were similar to those developed for the use of Sequenase with the robot except that all steps were performed for about five minutes and the elongation-labeling and termination steps were performed at 65 °C. It was therefore necessary to compensate for greater loss of volume due to evaporation (See Table). Denaturation was performed with a starting volume of 22 μ l. After annealing the volume was about 16 μ l and seven μ l of enzyme-labeling mix was added. Evaporation during extension resulted in loss of about seven μ l. Twenty μ l of water (that included the non-ionic detergents to 0.05% detergents) was added prior to dispensing seven μ l to each of the termination mixes. Evaporation resulted in a final volume of about seven μ l for each termination reaction. The reaction was stopped by the addition of five μ l STOP buffer.

Bst DNA polymerase gave excellent results and was stable in the reagent reservoir for at least 24 hours.

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Table
 Volumes (in μ l) of Sequencing Reactions
 During Different Segments

		<u>Sequenase</u>		<u>Bst DNA pol.</u>
		<u>Standard</u>	<u>Robot</u>	<u>Robot</u>
5	<u>Annealing Reaction</u>			
	Annealing mix	<u>10</u>	<u>16</u>	<u>22</u>
	Starting volume	10	16	22
10	[Evaporation]	<u>--</u>	<u>-6</u>	<u>-6</u>
		10	10	16
	<u>Labeling-Extension Reaction</u>			
	Enzyme-labeling mix added	<u>+5.5</u>	<u>+6</u>	<u>+7</u>
	Starting volume	15.5	16	23
15	[Evaporation]	<u>--</u>	<u>--</u>	<u>-7</u>
		15.5	16	16
	Volume added to compensate for evaporation	<u>-</u>	<u>9</u>	<u>20</u>
		15.5	25	36
20	Volume dispensed (total) per termination (4 times)	14	20	28
		3.5	5	7
	<u>Termination Reaction</u>			
	Termination mix	2.5	5	7
25	Dispensed elongation reaction	<u>+3.5</u>	<u>+5</u>	<u>+7</u>
		6	10	14
	[Evaporation]	<u>--</u>	<u>-2</u>	<u>-7</u>
		6	8	7
	<u>Addition of STOP Buffer</u>			
30	STOP Buffer	<u>+4.0</u>	<u>+4</u>	<u>+4</u>
	Final vol/termination reaction	10.0	12	11

The system according to the current invention could
 35 also be used in a diagnostic DNA sequencing laboratory, where
 speed, quality, and reproducibility are of paramount
 importance. Analysis of the sequences of the same gene or
 cDNA fragments from different patients makes it possible to
 produce rapidly large numbers of DNA sequencing templates

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especially if the PCR is employed. In addition, the demonstrated low-volume pipetting dexterity could be employed for sequencing with non-radioactive labels, as well. Use of fluorescent tags would accommodate those DNA sequencing
5 projects or facilities that utilize automated detection systems. Accordingly, the present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the
10 foregoing specification, as indicating the scope of the invention.

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CLAIMS

What is Claimed is:

1. A robotic system for automatically performing DNA sequencing, comprising:
 - 5 a) a robot controlled by a programmable controller and having an arm mounted for motion in vertical and horizontal planes;
 - b) first and second robot hands, said first hand being adapted to carry a microtiter plate, said second hand being adapted for pipetting, a coupling disposed on said robot arm for attaching each of said robot hands thereto;
 - 10 c) heating units adapted to hold a microtiter plate;
 - 15 d) a plurality of work stations, each adapted to support a microtiter plate;
 - e) a reagent reservoir holding unit containing a plurality of reservoirs; and
 - f) a controlled temperature storage unit.
- 20 2. The robotic system according to claim 1, wherein said second robot hand has a pipet tip, and wherein said programmable controller has logic for enabling said robot, with said second robot hand attached to said robot arm, to determine that said pipet tip has made contact with a
25 microtiter plate well bottom.
3. The robotic system according to claim 2,
~~wherein said robot further comprises means for determining~~
the force acting on said second hand, and wherein said logic comprises:
 - 30 a) means for instructing said robot to suspend said second hand above a microtiter plate by a predetermined height;
 - b) means for instructing said robot to decrease said height by a first predetermined increment;

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c) means for determining if said force is less than a predetermined value after said height of said second hand has been decreased by said first predetermined increment.

5 4. The robotic system according to claim 3,
wherein said logic further comprises:

a) means for instructing said robot to further decrease said height by a second predetermined increment; and

10 c) means for determining if said force is less than said predetermined value after said height of said second hand has been further decreased by said second predetermined increment;

whereby said second hand approaches a bottom of a microtiter
15 plate well by increasingly smaller decrements in said height of said second hand.

5. In a process of automatically sequencing DNA, a method of pipetting reagent solutions from microtiter plate wells using a pipet tip, comprising the steps of:

20 a) suspending said second hand above a microtiter plate by a predetermined height;

b) decreasing said height by a first predetermined increment;

25 c) measuring the force acting on said pipet tip after said height of said second hand has been decreased by said first predetermined increment;
and

d) determining if said force is less than a predetermined value.

30 6. The method according to claim 5, further comprising the steps of:

a) further decreasing said height by a second predetermined increment;

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b) measuring the force acting on said pipet tip after said height of said second hand has been decreased by said second predetermined increment; and

5 c) determining if said force is less than said predetermined value;

whereby said second hand approaches a bottom of a microtiter plate well by increasingly smaller decrements in said height of said second hand.

10 7. The method according to claim 6, further comprising the steps of:

a) further decreasing said height by a third predetermined increment;

15 b) measuring the force acting on said pipet tip after said height of said second hand has been decreased by said third predetermined increment; and

c) determining if said force is less than said predetermined value.

20 8. In a process of automatically performing nucleic acid sequencing in microtiter plate format employing sequencing reactions using reagent solutions and dideoxynucleotide terminations, a method of improving the pipettability of said reagents solutions, comprising the step
25 of placing non-ionic surfactants in said reagent solutions.

9. In a process of automatically performing nucleic acid sequencing employing sequencing reactions using reagent solutions, dideoxynucleotide terminations, and incubation, a method of compensating for the loss of volume
30 due to evaporation during said incubation, comprising the step of adding an aqueous solution to said sequencing reactions prior to said dideoxynucleotide terminations.

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10. The method according to claim 3, wherein said aqueous solution comprises a surfactant.

AMENDED CLAIMS

[received by the International Bureau
on 28 March 1994 (28.03.94);
original claim 4 cancelled; original claims 1-3, 10 amended;
new claims 11-31 added; other claims unchanged (8 pages)]

1. A robotic system for automatically performing DNA
5 sequencing, comprising:

- a) a robot having an arm mounted for motion in
vertical and horizontal planes;
- b) a hand for said robot arm having means for
pipetting a fluid from a microtiter plate well;
- 10 c) a microtiter plate having a well formed
therein, said well having a bottom, a reagent solution
contained in said well;
- d) a plurality of work surfaces, each having
means for supporting a microtiter plate;
- 15 e) a plurality of reservoirs, each having means
for holding a reagent;
- f) first storage means for storing microtiter
plates;
- 20 g) a programmable controller having first logic
means for instructing said robot to move said
pipetting means toward said microtiter plate well
bottom by a series of incremental movements, said
series of incremental movements including a set of
incremental movements in which each incremental
25 movement is successively smaller than each previous
incremental movement in said set.

2. The robotic system according to claim 1, wherein
said programmable controller has second logic means for
determining whether said pipetting means has made contact
30 with said microtiter plate well bottom after each of said
incremental movements in said set of movements.

3. The robotic system according to claim 2, wherein
said robot further comprises means for sensing a force acting
on said hand, and wherein said second logic means comprises:

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a) means for instructing said robot to sense an initial force acting on said hand prior to said set of incremental movements; and

5 b) means for determining if said force sensed differs from said initial force by more than a first predetermined value after each of said incremental movements in said set of movements.

5. In a process of automatically sequencing DNA, a
10 method of pipetting reagent solutions from microtiter plate wells using a pipet tip, comprising the steps of:

a) suspending said second hand above a microtiter plate by a predetermined height;

15 b) decreasing said height by a first predetermined increment;

c) measuring the force acting on said pipet tip after said height of said second hand has been decreased by said first predetermined increment; and

20 d) determining if said force is less than a predetermined value.

6. The method according to claim 5, further comprising the steps of:

a) further decreasing said height by a second predetermined increment;

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10. The method according to claim 9, wherein said aqueous solution comprises a surfactant.

11. The robotic system according to claim 1, wherein
5 said reagent solution contained in said well includes a non-ionic surfactant.

12. The robotic system according to claim 11, wherein said non-ionic surfactant in said reagent solution has a concentration of at least 0.02%.

10 13. The robotic system according to claim 12, wherein said non-ionic surfactant in said reagent solution has a concentration of about 0.02%.

14. The robotic system according to claim 1, further comprising means for holding and heating said microtiter
15 plate.

15. The robotic system according to claim 14, wherein said microtiter plate well defines a surface through which heat can flow into said well, and wherein said means for holding and heating said microtiter plate has a heat transfer
20 surface formed therein that conforms to said microtiter plate well surface.

16. The robotic system according to claim 14, wherein said pipetting means comprises a plurality of pipet tips capable of being coupled and uncoupled from said hand.

25 17. The robotic system according to claim 16, further comprising means for washing said pipet tips.

18. The robotic system according to claim 16, further comprising:

a) means for storing said pipet tips;

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- b) means for storing a plurality of empty microtiter plates; and
- c) means for heating said microtiter plate.

5 19. The robotic system according to claim 18, wherein said means for holding and heating said microtiter plate, said work surfaces, said reservoirs, and each of said storage means are arranged in a approximately circular array, and wherein said robot is centrally disposed within said array.

10 20. The robotic system according to claim 1, wherein said set of incremental movements comprises at least three incremental movements.

 21. The robotic system according to claim 2, wherein said programmable controller has third logic means for
15 instructing said robot to move said pipetting means away from said microtiter plate well bottom to allow aspiration of substantially all of said reagent solution from said microtiter plate well after said second logic means has determined that said pipetting means has made contact with
20 said microtiter plate well bottom.

 22. The robotic system according to claim 21, wherein said third logic means comprises means for instructing said robot to move said pipetting means in a direction away from said microtiter plate well bottom by a succession of
25 incremental movements until said means for determining whether said pipetting means is still in contact with said microtiter plate well bottom has determined that said pipetting means is not still in contact with said microtiter plate well bottom.

30 23. The robotic system according to claim 21, wherein said third logic means comprises:

- a) means for instructing said robot to move said pipetting means in a direction away from said

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microtiter plate well bottom by a first predetermined increment; and

5 b) means for determining whether said pipetting means is still in contact with said microtiter plate well bottom after said pipetting means has been moved away from said plate well bottom by said first predetermined increment.

24. The robotic system according to claim 23, wherein
10 said robot further comprises means for sensing a force acting on said hand, and wherein said means for determining whether said pipetting means is still in contact with said microtiter plate well bottom further comprises:

15 a) means for instructing said robot to sense an initial force acting on said hand prior to said movement by said first predetermined increment; and

 b) means for determining if said force sensed differs from said initial force by more than a first predetermined value after said pipetting means has
20 been moved by said first predetermined increment.

25 25. The robotic system according to claim 24, wherein said first predetermined value is approximately equal to the difference in said forced sensed when said hand is hanging freely from said robot arm immediately after said hand has been moved in a downward direction and said force sensed when
26 said hand is hanging freely from said robot arm immediately after said hand has been moved in an upward direction.

26. The robotic system according to claim 23,
wherein:

30 a) said first logic means further comprises means for instructing said robot to move said pipetting means toward said microtiter plate well bottom by at least one additional set of incremental

AMENDED SHEET (ARTICLE 19)

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5 movements, each incremental movement in any such additional sets being successively smaller than each previous incremental movement in its respective set, additional sets of incremental movements being performed in succession until said second logic means has determined that said pipetting means has contacted said microtiter plate well bottom; and

10 b) the value of said first predetermined increment is a function of the number of said sets of incremental movements performed.

27. The robotic system according to claim 2, wherein said first logic means further comprises means for instructing said robot to move said pipetting means toward
15 said microtiter plate well bottom by at least one additional set of incremental movements, each incremental movement in any such additional sets being successively smaller than each previous incremental movement in its respective set, additional sets of incremental movements being successively
20 performed until said second logic means has determined that said pipetting means has contacted said microtiter plate well bottom.

28. The robotic system according to claim 3, wherein said first predetermined value is approximately equal to the
25 difference in said forced sensed when said hand is hanging freely from said robot arm immediately after said hand has been moved in a downward direction and said force sensed when said hand is hanging freely from said robot arm immediately
after said hand has been moved in an upward direction.

30 29. A robotic system for automatically performing DNA sequencing, comprising:

a) a robot controlled by a programmable controller and having an arm mounted for motion in vertical and horizontal planes;

35 b) a plurality of microtiter plates each having a plurality of wells formed therein defining a

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5 surface through which heat can flow into said wells,
each of said wells having a bottom, a reagent
solution contained in at least a portion of said
wells;

10 c) first and second hands for said robot, said
first hand having means for carrying said microtiter
plates, said second hand having means for
simultaneously pipetting fluid from a plurality of
said microtiter plate wells;

d) means for supporting at least one of said
microtiter plates;

15 e) a heater for heating said microtiter plates,
said heater having a heat transfer surface formed
therein that conforms to said microtiter plate surface
defined by said wells; and

f) means for storing said microtiter plates.

30. The robotic system according to claim 29, further
20 comprising a force sensor for sensing a force applied to said
second hand, and wherein said programmable controller is
programmed with logic (i) to instruct said robot to bring
said second hand and one of said microtiter plates into
proximity so that said fluid pipetting means is suspended by
25 a predetermined height above said well bottoms of said one of
said microtiter plates, and then decrease said height by
which said fluid pipetting means is suspended above said well
bottoms in increasingly smaller increments and (ii) to
determine after each of said incremental decreases in height
30 if said force sensed has changed by more than a predetermined
amount, thereby indicating said second hand has contacted
said bottoms of said microtiter plate wells, and (iii) to
move said fluid pipetting means and said well bottoms apart
by a predetermined increment if said force sensed after any
35 of said incremental decreases in said height has changed by
more than said predetermined amount and (iv) to pipet said
reagent solution from said microtiter plate wells after said
fluid pipetting means and said well bottoms have been moved
apart by at least said predetermined increment.

AMENDED SHEET (ARTICLE 19)

31. A robotic system for automatically performing reactions in sequence, comprising:

- 5 a) means for containing a fluid, said fluid containing means having a bottom;
- b) a robot having means for drawing a fluid from said fluid containing means, said fluid drawing means normally spaced from said fluid containing means bottom by a distance;
- 10 c) means for causing relative motion between said fluid containing means and said fluid drawing means; and
- d) a programmable controller having logic means for instructing said relative motion causing means to
15 reduce said distance between said bottom of said fluid containing means and said fluid drawing means by a series of incremental movements, said series of incremental movements including a set of incremental movements in which each incremental movement is
20 successively smaller than each previous incremental movement in said set.

STATEMENT UNDER ARTICLE 19

The amendment to claims 1-3 has been made in order to more clearly distinguish these claims from the art cited in the International Search Report as being particularly relevant to the claimed invention. The amendment to claim 10 has been made to correct the dependency of the claim. The new claims 11-31 do not go beyond the disclosure of the international application as filed since full support for these claims may be found in the description of the preferred embodiment.

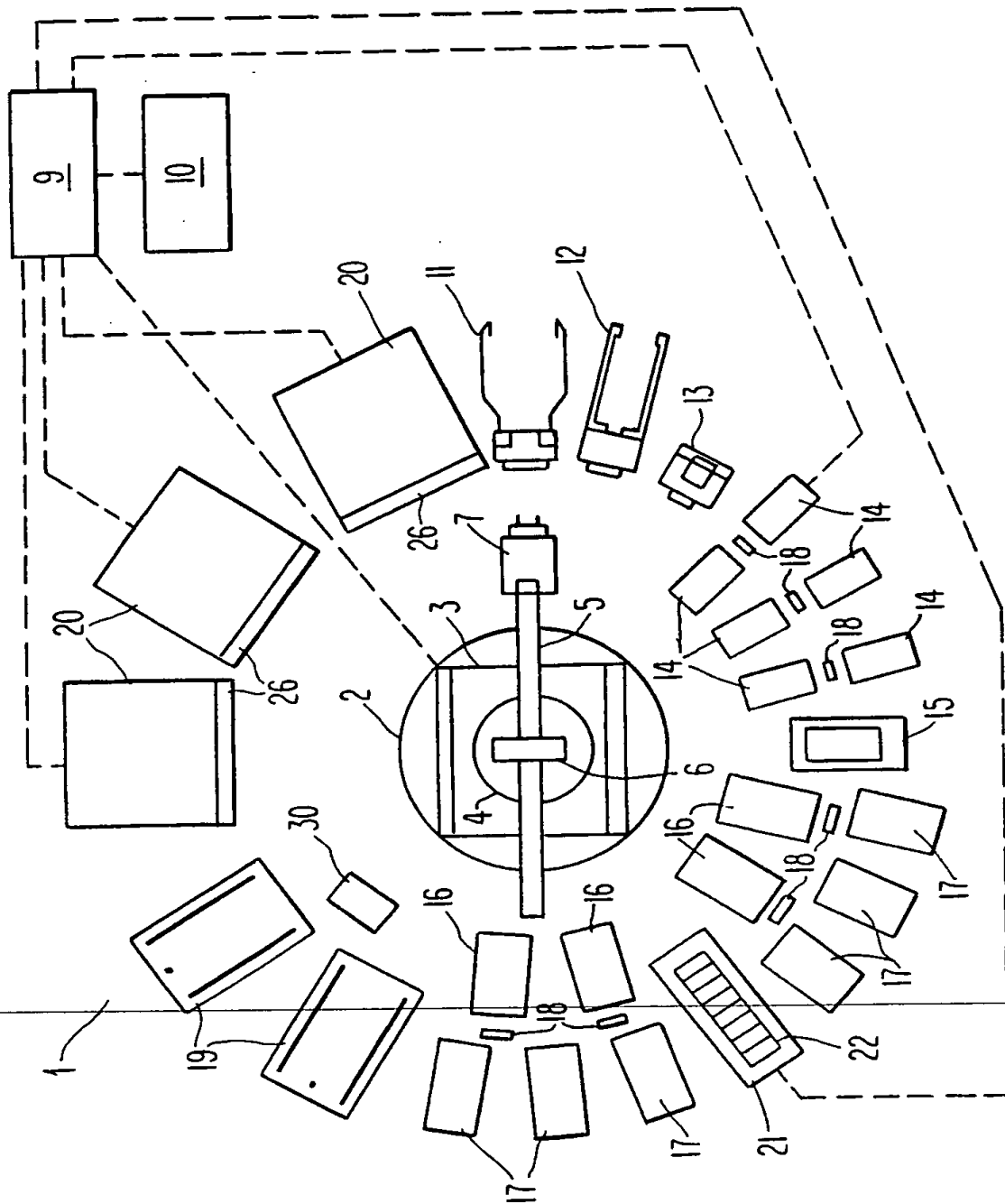


Fig. 1

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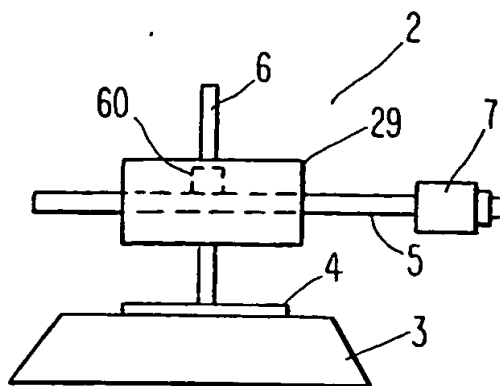


Fig. 2

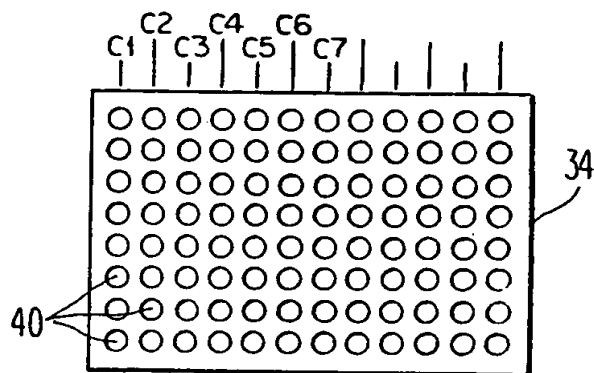


Fig. 7

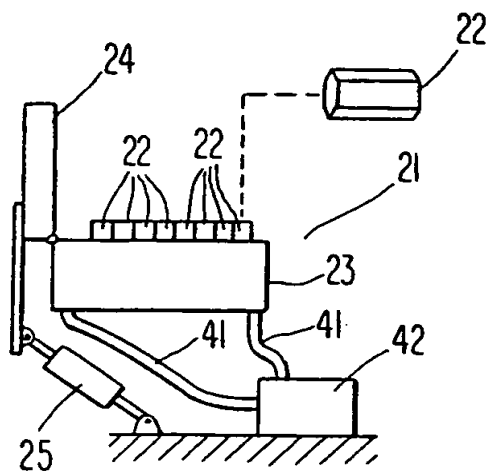


Fig. 3

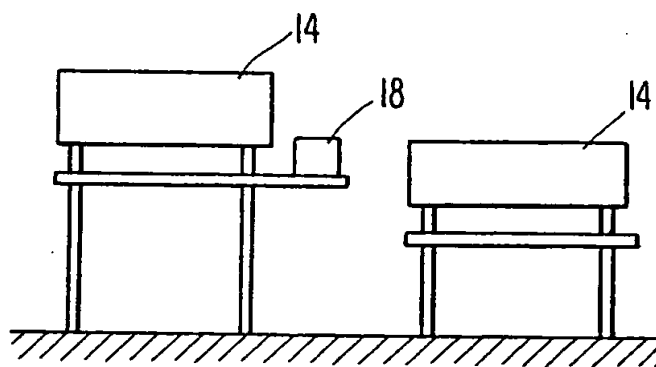


Fig. 4

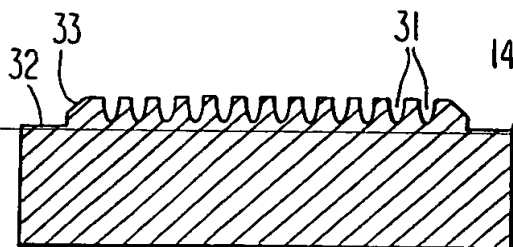


Fig. 5

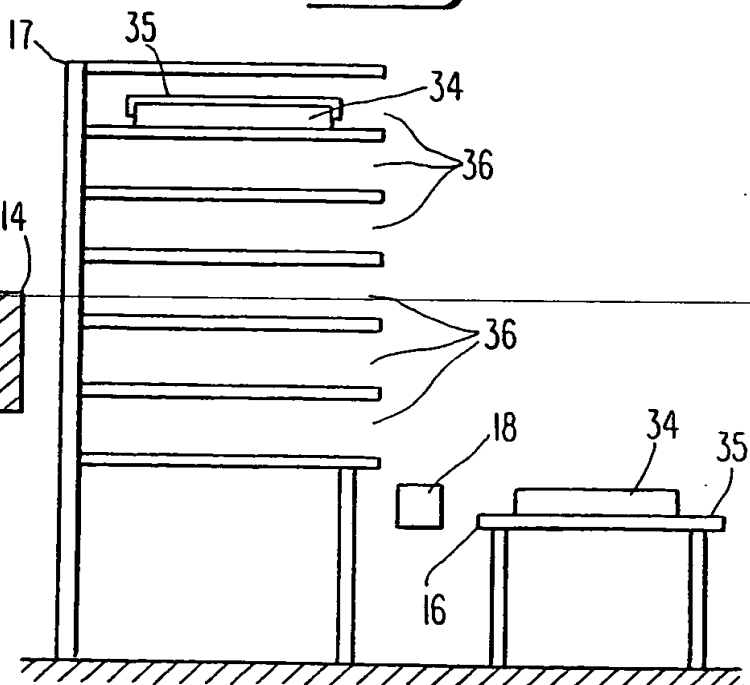


Fig. 6

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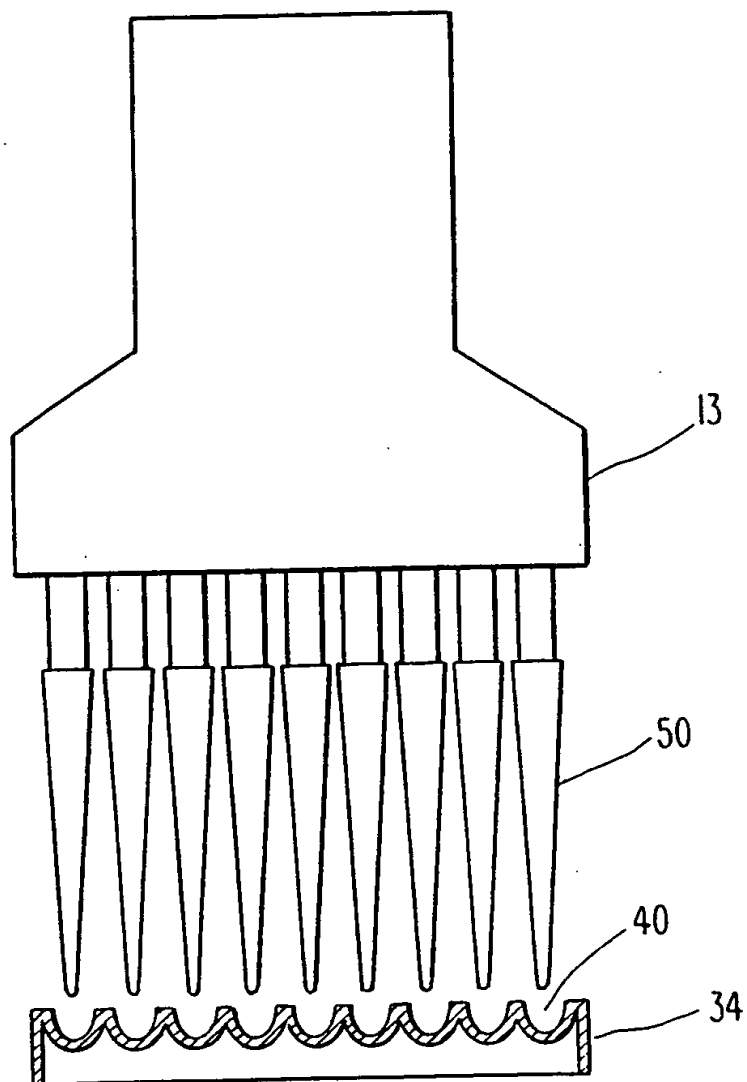


Fig. 8

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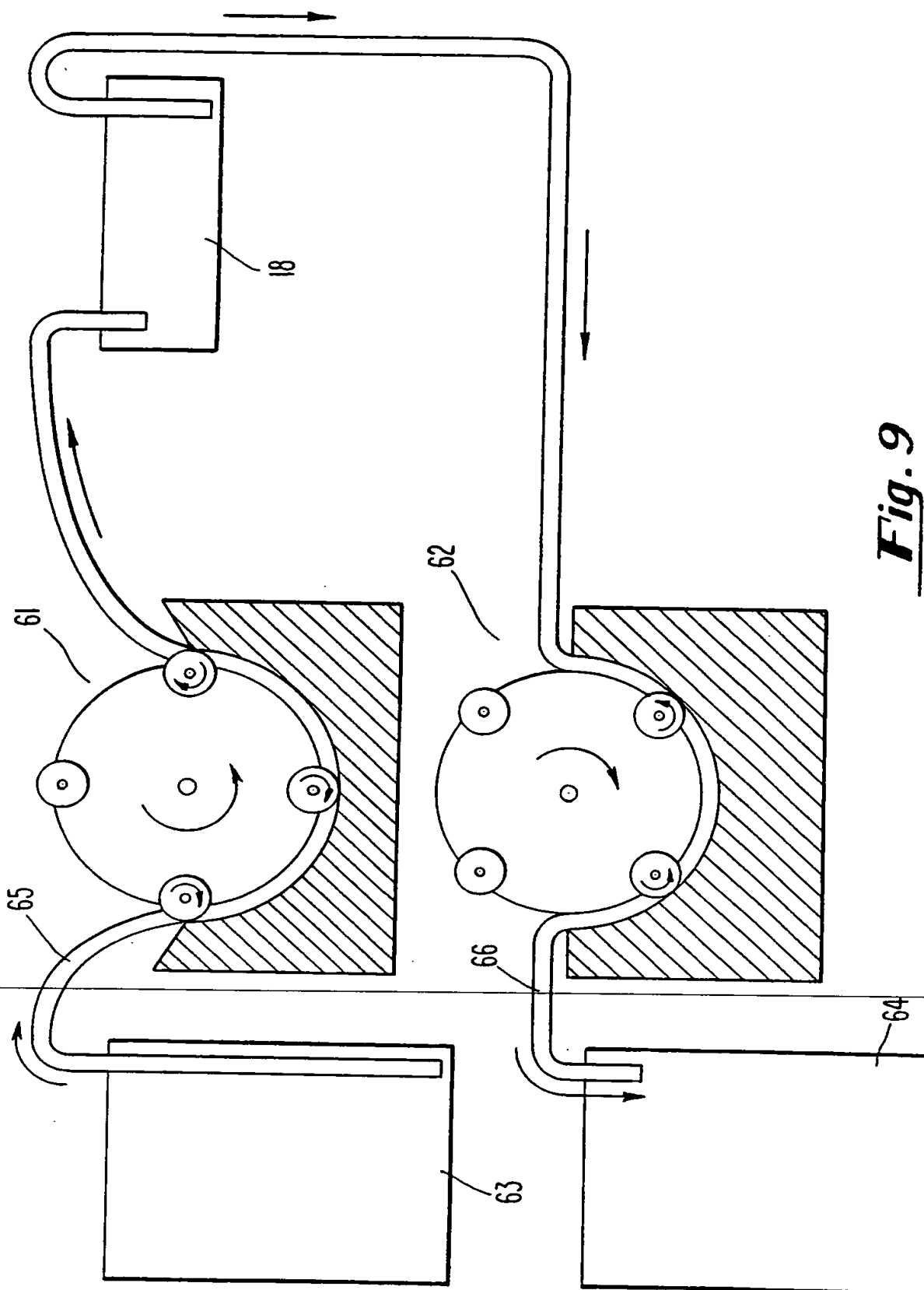
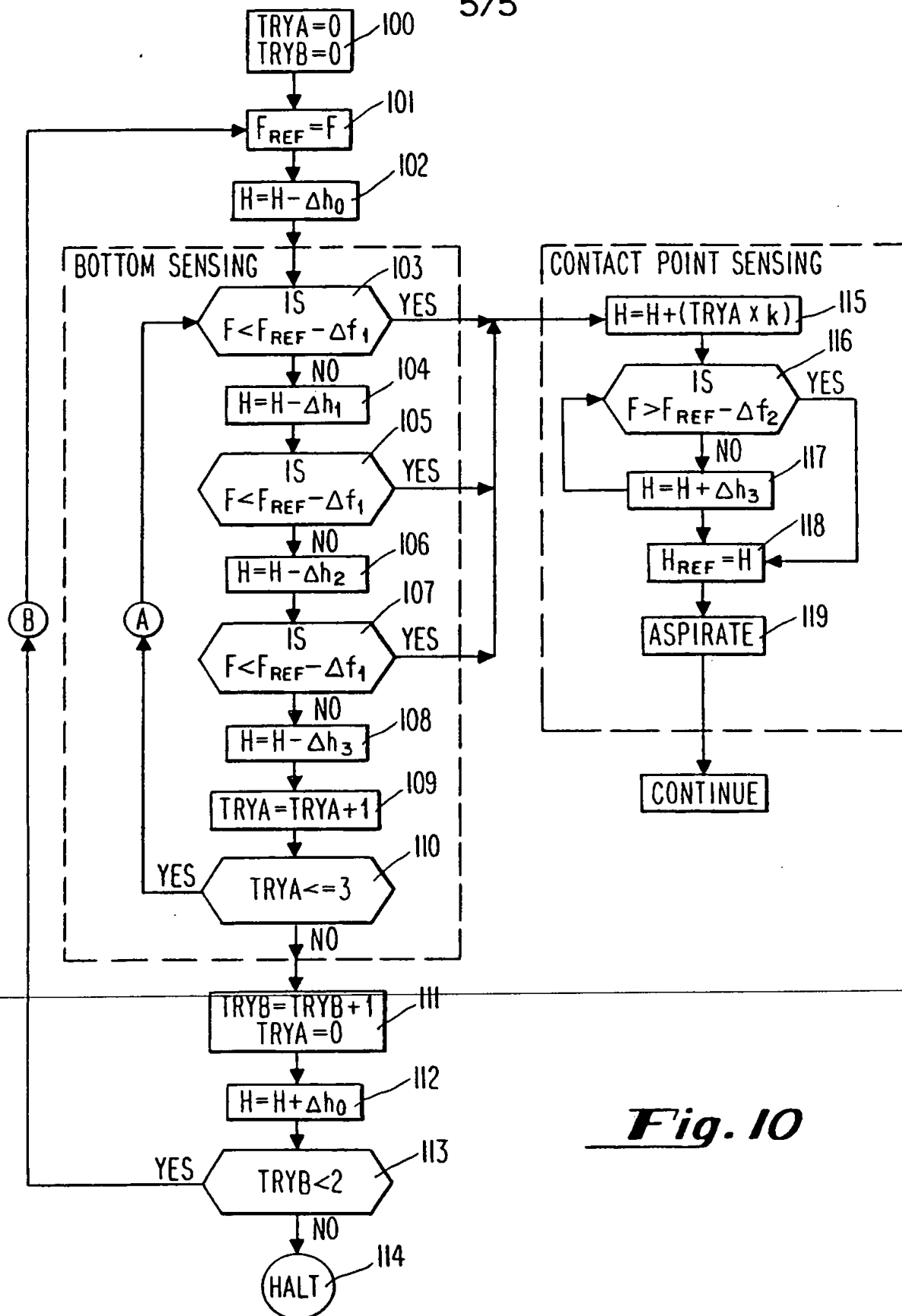


Fig. 9

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**Fig. 10**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09961

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) :B25J 9/00, 19/00, 19/02; B01L 3/02

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/63, 67, 100; 436/43, 174, 180; 73/863.01, 864.24, 864.25; 395/93; 901/10, 46; 318/568.16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: pipet#, pressure, force, surfactant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,715,413 (Backlund et al.) 29 December 1987, entire document, especially col. 4, line 41.	1-7
Y	US, A, 4,984,475 (Uffenheimer et al.) 15 January 1991, col. 16, lines 1-30.	8, 10
Y	WO, A, 91/16675 (Cathcart et al.) 31 October 1991, figures 1, 2a, 6c.	5-10
Y	Advances in Laboratory Automation Robotics, Zymark Corporation, issued 1987, S. Hamilton, "Robotic Assays for Fermentation Products", pages 1-21, see entire document.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Special categories of cited documents:		T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A	document defining the general state of the art which is not considered to be part of particular relevance		
E	earlier document published on or after the international filing date	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means		
P	document published prior to the international filing date but later than the priority date claimed	&	document member of the same patent family

Date of the actual completion of the international search

07 January 1994

Date of mailing of the international search report

FEB 03 1994

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09961

A. CLASSIFICATION OF SUBJECT MATTER: US CL :

422/63, 67, 100; 436/43, 174, 180; 73/863.01, 864.24, 864.25; 395/93; 901/10, 46; 318/568.16

